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CELLULAR AND MOLECULAR IMMUNOLOGY

THIRD EDITION

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teolytic enzyme papain preferentially cleaves rabbit IgG molecules into three separate pieces (Fig. 3–7). Two of the pieces are identical to each other and consist of an intact light chain associated with a V_H–Cγ1 fragment of the heavy chain. These fragments each retain the ability to bind antigen, a function of the V_L and V_H domains, and are therefore called **Fab** (fragment, antigen-binding). The third piece contains identical fragments of the γ heavy chain composed of the Cγ2 and Cγ3 domains. This piece of IgG has a propensity to self-associate and to crystallize into a lattice. It is therefore called **Fc** (fragment, crystalline). Lattice formation depends upon a uniformity of structure. The propensity of Fc regions to form a lattice reflects the presence of common amino acid sequences of the Cγ2 and Cγ3 domains shared by all antibodies of the same subtype. As we shall discuss later in this chapter, many of the effector functions of immunoglobulins are mediated by the Fc portions of the molecule. These proteolysis experiments provided the first evidence that the antigen recognition functions and the effector functions of Ig molecules are spatially segregated.

Different results are obtained when the proteolytic enzyme pepsin is used instead of papain to cleave rabbit IgG molecules (Fig. 3–7). In this case, under limiting conditions of enzyme concentrations and time, proteolysis is restricted to the carboxy terminus of the hinge region near the Cγ2 domain such that the antigen-binding fragment of IgG retains the hinge and the interchain disulfide bonds. Fab fragments retaining the heavy chain hinge are called Fab′; when the interchain disulfide bonds are intact, the two Fab′ fragments remain associated in a form called **F(ab′)$_2$**. The Fc fragment is often extensively degraded and does not survive proteolysis by pepsin. Fab and F(ab′)$_2$ are often useful as experimental tools because they can bind to antigens without activating Fc-dependent effector mechanisms.

These proteolysis experiments are not readily extended to other antibody isotypes such as IgM. In fact, they are not even applicable to all IgG

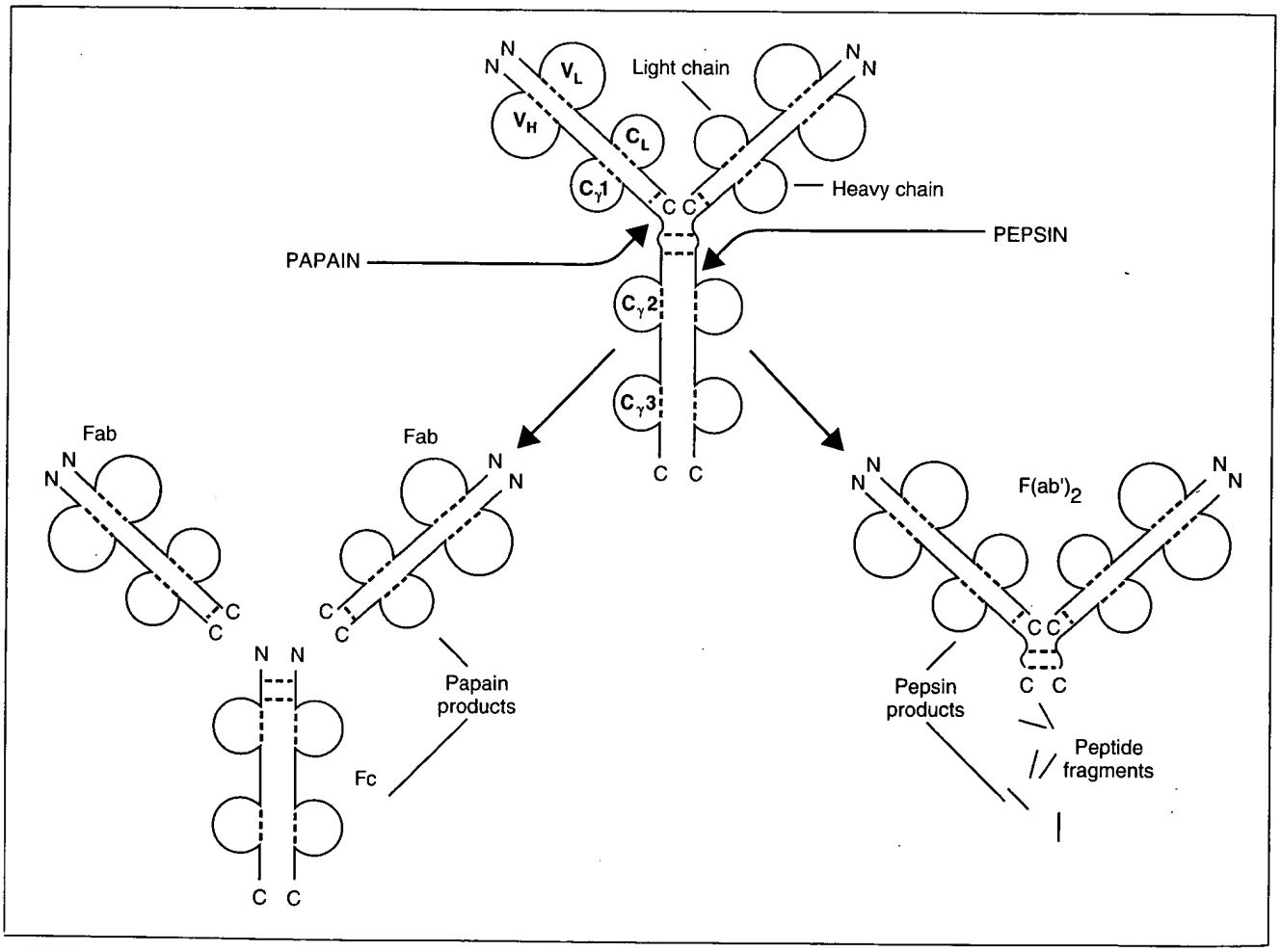

FIGURE 3–7. Proteolytic fragments of an immunoglobulin G (IgG) molecule. Sites of papain and pepsin cleavage are indicated by arrows. Papain digestion allows separation of two antigen-binding regions (the Fab fragments) from the portion of the IgG molecule that activates complement and binds to Fc receptors (the Fc fragment). Pepsin generates a single bivalent antigen-binding fragment [F(ab′)$_2$] with higher avidity for antigen than the two monovalent Fab fragments produced by papain cleavage.

1: Protein Eng. 1997 Apr;10(4):445-53. Related Articles, Link

Two amino acid mutations in an anti-human CD3 single chain Fv antibody fragment that affect the yield on bacterial secretion but not the affinity.

Kipriyanov SM, Moldenhauer G, Martin AC, Kupriyanova OA, Little M.

Department of Molecular Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Recombinant antibody fragments directed against cell surface antigens have facilitated the development of novel therapeutic agents. As a first step in the creation of cytotoxic immunoconjugates, we constructed a single-chain Fv fragment derived from the murine hybridoma OKT3, that recognizes an epitope on the epsilon-subunit of the human CD3 complex. Two amino acid residues were identified that are critical for the high level production of this scFv in Escherichia coli. First, the substitution of glutamic acid encoded by a PCR primer at position 6 of VH framework 1 by glutamine led to a more tha a 30-fold increase in the production of soluble scFv. Second, the substitution of cysteine by a serine in the middle of CDR-H3 additionally doubled the yield of soluble antibody fragment without any adverse effect on its affinity for the CD3 antigen. The double mutant scFv (Q,S) proved to be very stable in vitro: no loss of activity was observed after storage for 1 month at 4 degrees C, while the activity of scFv containing a cysteine residue in CDR-H3 decreased by more than half. The results of production yield, affinity, stability measurements and analysis of three-dimensional models of the structure suggest that the sixth amino acid influences the correct folding of the VH domain, presumably by affecting a folding intermediate, but has no effect on antigen binding.

PMID: 9194170 [PubMed - indexed for MEDLINE]

1: Immunology. 1982 Mar;45(3):473-81.

Studies on the possible involvement of complement component C3 in the initiation of acid hydrolase secretion by macrophages.

Riches DW, Morris CJ, Stanworth DR.

Complement component C3 has been detected on the plasma membranes of mouse peritoneal macrophages by using an immunoperoxidase technique in conjunction with transmission electron microscopy. Evidence that such cell surface-associated C3 might be the trigger for lysosomal enzyme discharge was sought initially by exposing macrophage monolayers to anti-mouse C3 F (ab')2, and later, by treating cells with the antibody fragment before two potent secretagogues (methylamine and zymosan particles). Both methods, however, failed to demonstrate a role for cell surface-associated C3 in the initiation of enzyme secretion.

PMID: 7037623 [PubMed - indexed for MEDLINE]

Targeted gene delivery by tropism-modified adenoviral vectors.

Douglas JT, Rogers BE, Rosenfeld ME, Michael SI, Feng M, Curiel DT.

Gene Therapy Program, University of Alabama at Birmingham 35294, USA.

The utility of adenoviral vectors for gene therapy is currently limited due, in part, to the widespread distribution of the cellular receptor for the adenovirus fiber that precludes the targeting of specific cell types. In order to develop a targeted adenovirus, it is therefore necessary both to ablate endogenous viral tropism and to introduce novel tropism. We hypothesized that these two goal could be achieved by employing a neutralizing anti-fiber antibody, or antibody fragment, chemically conjugated to a cell-specific ligand. To test this concept, we chose to target the folate receptor, which is overexpressed o the surface of a variety of malignant cells. Therefore, we conjugated folate tc the neutralizing Fab fragment of an anti-fiber monoclonal antibody. This Fab folate conjugate was complexed with an adenoviral vector carrying the luciferase reporter gene and was shown to redirect adenoviral infection of target cells via the folate receptor at a high efficiency. Furthermore, when complexed with an adenoviral vector carrying the gene for herpes simplex virus thymidine kinase, the Fab-folate conjugate mediated the specific killing of cells that overexpress the folate receptor. This work thus represents the first demonstration of the retargeting of a recombinant adenoviral vector via a non-adenoviral cellular receptor.

PMID: 9634824 [PubMed - indexed for MEDLINE]

1: J Exp Med. 1981 Jun 1;153(6):1615-28. Related Articles, Link

Membrane distribution and adsorptive endocytosis by C3b receptors on human polymorphonuclear leukocytes.

Fearon DT, Kaneko I, Thomson GG.

C3b receptors on human polymorphonuclear leukocytes (PMN) were nonrandomly distributed in small clusters on the plasma membranes of these cells when assessed by indirect immunofluorescence at 0 degree C using monospecific rabbit Fab' or F(ab')2 anti-C3b receptor and tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat IgG anti-F (ab')2. When PMN were incubated with the bivalent anti-C3b receptor at 37 rather than at 0 degree C, almost no immunofluorescence was observed, which indicates that the C3b receptor-F(ab')2 complexes had been rendered inaccessible to TRITC-IgG anti-F(ab')2. Endocytosis of the anti-C3b recepto ligand was quantitated by measuring the binding 131I-IgG anti-F(ab')2 by PMN that had previously taken up 125I-F(ab')2 anti-C3b receptor at 0 and at 37 degree C, respectively. There was a constant 2: 1 molar ratio of anti-F(ab' 2 to anti-C3b receptor with PMN that had been incubated with the first antibody at 0 degree C. In contrast, when increments of F(ab')2 anti-C3b receptor were taken up by the cells at 37 degree C, there was a dose-related decline in this molar ratio to a minimum of 0.2 molecules of anti-F(ab')2 anti F(ab')2 bound per molecule of PMN-associated anti-C3b receptor. 125I-F(ab 2 anti-C3b receptor taken up by PMN at 37 degree C was also inaccessible tc release by proteolytic treatment of the cells with pronase. The rate of endocytosis of 125I-F(ab')2 anti-C3b receptor was rapid as the PMN-bound antibody fragment became inaccessible to 131I-IgG anti-F(ab')2 within 10 min during incubation of the cells at 37 degree C. In contrast to these findings, 125I-Fab' anti-C3b receptor that was taken up by PMN at 37 degree C remained accessible to both 131I-IgG anti-F(ab')2 and to proteolytic releas by pronase, which suggests that monovalent interaction of ligand with C3b receptors was not sufficient for induction of endocytosis. The requirement fo multivalency was also demonstrated using the C3b-OR, the normal ligand fo the C3b receptor. 125I-C3b-OR was specifically bound by PMN but remained on the cell receptor. 125I-C3b-OR was specifically bound by PMN but remained on the cell surface, as determined by its accessibility to pronase unless it was cross-linked with F(ab')2 anti-C3. Although C3b receptors on PMN do not mediate internalization of adsorptive pinocytosis of soluble ligand indicates their potential for the clearance of C3b-bearing immune

complexes without recruitment of other cell surface receptors.

PMID: 7252422 [PubMed - indexed for MEDLINE]

Indirect antiproliferative effect of the somatostatin analog octreotide on MIA PaCa-2 human pancreatic carcinoma in nud mice.

Weckbecker G, Raulf F, Bodmer D, Bruns C.

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Analogs of somatostatin (SRIF) such as octreotide exert antiproliferative effects that are mediated directly by tumoral SRIF receptors or indirectly by down-modulation of factors that stimulate tumor growth. Direct and indirect antiproliferative effects have been demonstrated in certain SRIF receptor-positive and -negative human breast cancer models in nude mice, respectively. These antiproliferative mechanisms are also being explored in other cancer types including pancreatic cancer. While clinical pilot studies have indicated that a fraction of pancreatic adenocarcinomas respond to high dose octreotide treatment, it is known from receptor autoradiographic and scintigraphic studies that human pancreatic carcinomas fail to express SRIF receptors, in contrast to rat pancreatic carcinomas or human endocrine pancreatic cancer. Studies on the potential anticancer effect of octreotide on the growth of experimental human pancreatic cancer and its SRIF receptor status have been controversial. Therefore, we investigated in vivo the effects of octreotide on the growth of MIA PaCa-2 human pancreatic carcinomas raised from cultured cells with a low passage number after receipt from the American Type Culture Collection. Nude mice bearing MIA PaCa-2 tumors were treated with a single injection of the recently developed octreotide long acting release formulation, "SMS pa LAR." This treatment was well tolerate and resulted in a highly significant inhibition of tumor growth during weeks three and eight after administration. MIA PaCa-2 tumors were removed after eight weeks and processed for RT-PCR analysis using probes specific for each of the five somatostatin receptor subtypes sst1-sst5. This analysis revealed that MIA PaCa-2 tumors, like human pancreatic adenocarcinomas, do not express any of the five SRIF receptor subtypes, suggesting an indirect mode of tumor growth inhibition. In summary, the depot formulation SMS p LAR exerted long-lasting antiproliferative effects in SRIF receptor-negative human pancreatic carcinomas in nude mice.

1: Front Neuroendocrinol. 1993 Jan;14(1):27-55.

Octreotide and related somatostatin analogs in the diagnosis anc treatment of pituitary disease and somatostatin receptor scintigraphy.

Lamberts SW, Hofland LJ, de Herder WW, Kwekkeboom DJ, Reubi JC Krenning EP.

Department of Medicine, Erasmus University, Rotterdam, The Netherlands.

Clinical introduction of octreotide, a long-acting somatostatin analog, has opened a new era in the medical therapy of patients with growth hormone (GH)- and thyroid-stimulating hormone (TSH)-secreting pituitary tumors. Good control of hormonal hypersecretion occurred in most patients, and tumor shrinkage has been observed in more than half of them. Octreotide therapy is of no value in most patients with Prolactin (PRL)- and adrenocorticotrophic (ACTH)-secreting pituitary tumors. However patients with Cushing's syndrome caused by ectopic ACTH secretion from a variety c endocrine tumors benefit from octreotide administration. In patients with visual disturbances related to chiasmal compression by nonfunctioning pituitary tumors, somatostatin analog administration has been reported to result in rapid improvement in visual acuity. This beneficial effect might not be related to a direct action of octreotide, but may reflect an effect on the retina and/or optic nerve. The presence of somatostatin receptors on a wide variety of pituitary tumors as well as on a number of parasellar tumors allow: their in vivo visualization with radionucleotide-labelled somatostatin analogs A positive scan in patients with GH- and TSH-secreting pituitary tumors is predictive of a good suppressive effect of octreotide on hormone release by these tumors. PRL- and ACTH-secreting pituitary adenomas cannot be visualized, but clinically nonfunctioning pituitary adenomas are visualized in 75% of cases with 111In-DTPA-octreotide. At present it is unclear whether this has consequences with regard to the medical treatment of these last grou of patients. Somatostatin receptor scintigraphy can be successfully used in th differential diagnosis between pituitary hypersecretion of GH and/or ACTH and the ectopic secretion of growth hormone-releasing hormone (GHRH) anc ACTH by peripherally localized endocrine tumors. Again the visualization o such tumors also predicts successful control of hormonal hypersecretion by octreotide.

1: J Pept Res. 1998 Jan;51(1):55-64. Related Articles, Linl

Biological activity and three-dimensional structure of an agonist analog of bombesin.

Condamine E, Chapdeleine G, Demarcy L, Duclos JF, Davoust D, Llinares M, Azay J, Martinez J, Chapelle S.

Institut Federatif de Recherche Multidisciplinaire sur les Peptides, UPRESA CNRS 6014, UFR des Sciences, Universite de Rouen, Mont-Saint-Aignan, France.

JMV635, a nonapeptide analog of the active terminal nonapeptide segment o bombesin, was tested for its ability to stimulate in vitro amylase release from rat pancreatic acinar cells and to inhibit the binding of gastrin-releasing peptide to rat pancreatic acini. It was found to be a full agonist of bombesin and to recognize the bombesin receptor with moderate potency. The NMR proton assignments of JMV635 were achieved, and the conformations of JMV635 in aqueous solution and in trifluoroethanol at 297 K were determined using two-dimensional COSY, HOHAHA, NOESY and ROESY experiments. In trifluoroethanol, JMV635, like the active part of bombesin, showed a partial alpha-helical structure. These results were confirmed by circular dichroism and refined by restrained molecular dynamic methods. Structure calculations, using the distance and angle restraints obtained from NMR data on JMV635, gave a total of 75 structures which could be aligned t a root mean square deviation of the bond length of 0.007 A and of the valenc angle of 1.55 degrees for the backbone atoms of the amino acid residues. The conformation is a well-defined right-handed alpha-helix in the C-terminal Q2 G6 segment and is less structured in the three C-terminal residues.

PMID: 9495592 [PubMed - indexed for MEDLINE]

1: Prostate. 1992;20(4):269-80. Related Articles, Linl

Inhibition of growth of PC-82 human prostate cancer line xenografts in nude mice by bombesin antagonist RC-3095 or combination of agonist [D-Trp6]-luteinizing hormone-releasing hormone and somatostatin analog RC-160.

Milovanovic SR, Radulovic S, Groot K, Schally AV.

Endocrine Institute, Veterans Administration Medical Center, New Orleans, LA 70146.

The effects of treatment with a bombesin receptor antagonist [D-Tpi6, Leu13 psi (CH2NH) Leu14]BN(6-14)(RC-3095) and the combination of an agonist of luteinizing hormone-releasing hormone [D-Trp6]-LH-RH and somatostati analog D-Phe-Cys-Tyr-D-Trp-Lys-Val- Cys-Trp-NH2 (RC-160) were studie in nude mice bearing xenografts of the hormone-dependent human prostate tumor PC-82. During the 5 weeks of treatment, tumor growth was decreased in all treated groups compared with controls. Bombesin antagonist RC-3095 and the combination of [D-Trp6]-LH-RH and RC-160 caused a greater inhibition of tumor growth than [D-Trp6]-LH-RH or RC-160 alone as based on measurement of tumor volume and percentage change in tumor volume. The largest decrease in tumor weight was also seen in the groups treated with the bombesin antagonist and with the combination of RC-160 and [D-Trp6]-LH-RH. Serum prostatic-specific antigen levels were greatly decreased, and insulin-like growth factor I (IGF-I) as well as growth hormone levels were reduced in all treated groups. Specific binding sites for [D-Trp6]-LH-RH, epidermal growth factor (EGF), IGF-I, and somatostatin (SS-14) were found in the tumor membranes. Receptors for EGF were significantly down-regulated by treatment with the bombesin antagonist or RC-160. Combinatio of LH-RH agonists with somatostatin analog RC-160 might be considered fo improvement of hormonal therapy for prostate cancer. The finding that bombesin antagonist RC-3095 inhibits the growth of PC-82 prostate cancer suggests the merit of further studies to evaluate the possible usefulness of antagonists of bombesin in the management of prostatic carcinoma.

PMID: 1376910 [PubMed - indexed for MEDLINE]

1: Biochem J. 1987 Oct 15;247(2):427-32. Related Articles, Linl

C-terminal bombesin sequence requirements for binding and effects on protein synthesis in Swiss 3T3 cells.

Gargosky SE, Wallace JC, Upton FM, Ballard FJ.

Department of Biochemistry, University of Adelaide, South Australia.

1. Synthetic peptides corresponding to the five, seven, nine and eleven C-terminal amino acids of the tetradecapeptide bombesin as well as bombesin itself and gastrin-releasing peptide have been evaluated in Swiss 3T3 cells in order to define the minimal peptide length needed for biological responsiveness. 2. Gastrin-releasing peptide, bombesin, the undecapeptide and nonapeptide had nearly equipotent abilities to compete for binding of labelled gastrin-releasing peptide to the cell receptors and showed half-maximal competition at 5-10 nM. The heptapeptide and pentapeptide were ineffective. 3. Cross-linking experiments demonstrated specific binding of gastrin-releasing peptide to a 100 kDa receptor subunit. 4. Total cell protein synthesis was stimulated equally by the nonapeptide and longer peptides witl a half-maximal effect at 0.5 nM, while a more than 1000-fold higher concentration of the heptapeptide was required to produce a similar response Comparable results were found when insulin was also present. 5. Neither an inhibition of protein breakdown nor a stimulation of DNA labelling could be demonstrated by bombesin or gastrin-releasing peptide. 6. We conclude that C-terminal peptide ligand comprising more than seven but no more than nine amino acids is required to achieve high-affinity binding and receptor-mediated responses via the bombesin receptor.

PMID: 3426545 [PubMed - indexed for MEDLINE]

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In vitro and in vivo studies of substance P receptor expression ir rats with the new analog [indium-111-DTPA-Arg1]substance P.

Breeman WA, VanHagen MP, Visser-Wisselaar HA, van der Pluijm ME Koper JW, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Hazenberg MP, Lamberts SW, Visser TJ, Krenning EP.

Department of Nuclear Medicine, University Hospital Dijkzigt, Rotterdam, The Netherlands.

We evaluated the potential usefulness of a new radiolabeled substance P (SP) analog, [111In-DTPA-Arg1]SP, as a radiopharmaceutical for the in vivo detection of SP receptor-positive (SPR+) immunologic disorders (i.e., inflammatory bowel disease and arthritis) and tumors (i.e., carcinoid). METHODS: Substance P, [DTPA-Arg1]SP and [3-(p-hydroxyphenyl) propionyl-Arg1]SP (Bolton-Hunter-SP, [BH-SP]) were tested as competitors for 125I-BH-SP to SPR in rat brain cortex membranes. An autoradiographic displacement study of the submandibular gland of the rat with the 125I-BH-SP as radioligand and [DTPA-Arg1]SP as competitor was performed. Tissue distribution and ex vivo autoradiography were studied in rats, with and without pretreatment with the selective nonpeptide antagonist CP96,345 to quantify specific binding. In vivo metabolism of [111In-DTPA-Arg1]SP was performed in control rats. Gamma-camera scintigraphic studies were carried out with control rats to visualize the SPR+ salivary glands in rats bearing the SPR+ transplantable pancreatic tumor CA20948 and in rats with SPR+ adjuvant arthritic joints, which was induced after injection of a homogenate c Mycobacterium tuberculosis. RESULTS: Substance P, [DTPA-Arg1]SP and BH-SP dose-dependently inhibited binding of 125I-BH-SP to SPR in rat brai cortex membranes with IC50 values of 0.2, 4 and 2 nM, respectively. In an autoradiographic displacement study of the submandibular gland with 125I-BH-SP as radioligand, an IC50 of 2.7 nM was found for [DTPA-Arg1]SP. In vivo metabolism of the radiopharmaceutical in the rat revealed a renal clearance rate of 50% of the injected radioactive dose in 30 min and a rapid enzymatic degradation of the radiopharmaceutical, resulting in an effective half-life of the intact radiopharmaceutical in blood of approximately 3 min. Tissue distribution and ex vivo autoradiographic studies in rats showed uptake and specific binding of radioactivity in isolated tumors and submandibular and parotid glands. Optimum SPR+ target-to-background

ratios were found 24 hr after injection of [111In-DTPA-Arg1]SP. Visualization of normal SPR+ tissues, such as the salivary glands by gamma camera scintigraphy, after administration of [111In-DTPA-Arg1]SP was demonstrated in untreated rats. Pathological SPR+ processes were visualized both in rats bearing the transplantable pancreatic tumor CA20948 and in thos with adjuvant mycobacteria tuberculosis-induced arthritic joints. CONCLUSION: [Indium-111-DTPA-Arg1]SP can be used successfully to visualize SPR+ processes in vivo by gamma camera scintigraphy.

PMID: 8543978 [PubMed - indexed for MEDLINE]

1: Biochemistry. 1997 Apr 15;36(15):4542-51. Related Articles, Linl

ACS PUBLICATIONS

p-(4-Hydroxybenzoyl)phenylalanine: a photoreactive amino acic analog amenable to radioiodination for elucidation of peptide-protein interaction. Application to substance P receptor.

Wilson CJ, Husain SS, Stimson ER, Dangott LJ, Miller KW, Maggio JE.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Massachusetts General Hospital, Boston 02115, USA.

Benzoylphenylalanine, a photoreactive phenylalanine analog that can be incorporated into a peptide during solid-phase synthesis, is a useful probe for investigating the interactions of bioactive peptides with their receptors. This probe, however, lacks versatility because it is not detectable by Edman sequencing and because it cannot be labeled with radioiodine, requiring radiolabeling of the peptide ligand at a site distal to the photoreactive amino acid. The separation of the radioisotope and photoaffinity labels along the primary sequence limits identification of the photoinsertion site to a peptide fragment rather than a specific amino acid of the receptor protein. We have now synthesized p-(4-hydroxybenzoyl)phenylalanine by a synthetic route involving reaction of 4-(chloromethyl)benzoic anhydride with phenol in polyphosphoric acid to give the 4-(chloromethyl)benzoyl ester of 4-(chloromethyl)-4'-hydroxybenzophenone followed by reaction of the benzophenone derivative with ethyl acetamidocyanoacetate and subsequent hydrolysis of the product to give p-(4-hydroxybenzoyl)phenylalanine. The novel photolabile amino acid was incorporated into substance P (replacing Phe8 or Lys3) to give 11-mer peptides that bind with high (nM) affinity and specificity to the substance P receptor. Radioiodination of the substance P analogs resulted in the incorporation of 125I at the photoreactive amino acid residue, yielding probes of high (approximately 2000 Ci/mmol) specific activity. Subsequent photolysis of the radiolabeled peptides in the presence o substance P receptor caused covalent attachment of the peptide to the receptc with high photoinsertion yield (approximately 30%); photolabeling was abolished in the presence of excess unlabeled SP. p-(4-Hydroxybenzoyl) phenylalanine retains p-benzoylphenylalanine's high insertion yield and low reactivity with water, but in contrast allows placement of radioiodine and the photoactive moieties within the same residue, providing the ability to identif the specific site(s) of interaction, and identification of the residue by Edman

sequencing. This novel amino acid may be useful in the elucidation of the interaction of a variety of peptides with their receptors.

PMID: 9109663 [PubMed - indexed for MEDLINE]

1: J Med Chem. 1995 Jan 6;38(1):137-49.

Ac-[3- and 4-alkylthioproline31]-CCK4 analogs: synthesis and implications for the CCK-B receptor-bound conformation.

Kolodziej SA, Nikiforovich GV, Skeean R, Lignon MF, Martinez J, Marshall GR.

Department of Molecular Biology and Pharmacology, Washington Universit School of Medicine, St. Louis, Missouri 63110.

It has been reported that substitution of the Met31 residue in Boc-CCK4 (Boc-Trp30-Met31-Asp32-Phe33-NH2, CCK33 numbering) by trans-3-propyl-L-proline yields a highly potent and selective CCK-B agonist. To further explore the structural requirements of the Met31 side chain in the receptor-bound conformation of CCK4, we have synthesized several Ac-CCK4 analogs containing substitution of Met31 by 3- and 4-(alkylthio)-substituted proline derivatives. To this end we have developed novel syntheti routes to enantiomerically pure N-Boc-4-cis- and -trans-(methylthio)prolines and racemic N-Boc-3-cis and -trans-[(4-methylbenzyl)thio]prolines. The protected mercaptoprolines were incorporated into Ac-CCK4 analogs using SPPS and were alkylated using various electrophiles following cleavage fron the solid support. Binding assays reveal that 3-(alkylthio)prolines analogs have higher affinities at the CCK-B receptor than the corresponding 4-(alkylthio)proline analogs, and that trans-3-(alkylthio)proline analogs had higher affinities than corresponding cis-3-(alkylthio)proline analogs. Within both the cis- and trans-3-(alkylthio)proline series, the order of potency was found to be Me < Et < n-Pr. The trans-3-(n-propylthio)-L-proline analog demonstrates a higher affinity than that reported for Boc-CCK4[trans-3-propyl-L-Pro31]. Comparison of the low-energy structures calculated for several high-affinity Ac-CCK4 analogs reveal a common geometry which we propose to be the CCK-B receptor-bound conformation. This model shows grouping of the hydrophobic side chains of Trp, Met, and Phe at one side of the molecule and the hydrophilic side chain of Asp and the C-terminal carboxamide at the other side.

PMID: 7837225 [PubMed - indexed for MEDLINE]

1: Brain Res. 1998 May 11;792(2):246-52. Related Articles, Linl

Evidence for additional neurotensin receptor subtypes: neurotensin analogs that distinguish between neurotensin-mediated hypothermia and antinociception.

Tyler BM, Cusack B, Douglas CL, Souder T, Richelson E.

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Neurotensin (NT), a tridecapeptide, is a neurotransmitter that elicits potent effects including hypothermia and antinociception in mice and rats. To date, there are two types of the neurotensin receptor (NTR) that have been molecularly cloned from the rat. However, several lines of evidence suggest the presence of additional NTR subtypes. We have identified a NT analog of the NT(8-13) fragment, NT27, that selectively causes only the hypothermic response in vivo, when microinjected into the periaqueductal gray (PAG) of rats. A dose of 18 nmol of NT or NT27 caused a body temperature lowering of 1.8 and 1.2 degrees C, respectively. This same dose of NT or NT27 yielde a hotplate maximum physiological effect of 75% and 25%, respectively. Interestingly, despite its high KD (620 nM) at the cloned NTR-1, NT27-I (th iodinated form of NT27) exerted a potent hypothermic effect even at a very low dose (0.6 nmol). Equally intriguing, was that NT24, a sterioisomer of NT27, with a much higher affinity (KD=0. 5 nM) at NTR-1, did not selectively induce hypothermia in mice, but did selectively induce hypothermia in rats. Copyright 1998 Elsevier Science B.V.

PMID: 9593920 [PubMed - indexed for MEDLINE]

1: Arch Biochem Biophys. 1996 Oct 1;334(1):151-7. Related Articles, Linl

ELSEVIER FULL-TEXT ARTICLE

Processing of carcinoembryonic antigen by Kupffer cells: recognition of a penta-peptide sequence.

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Carcinoembryonic antigen (CEA) binds to an 80-kDa cell surface receptor or Kupffer cells via the peptide sequence PELPK (residues 108-112) located at the hinge region between the N and Al immunoglobulin-like domains. This study is aimed at analyzing the specificity of the peptide binding, determinin biodistribution of 80-kDa receptor, and processing of CEA by this receptor. We synthesized a number of bovine serum albumin (BSA) derivatives carrying PELPK and related sequences. A series of peptides (YPELPK, YPDLPK, YPDLPR, and YPELGK) were conjugated to bovine serum albumin using N-hydroxysuccinimidyl-4-azidobenzoate. When 125I peptide conjugates, CEA, and BSA were injected intravenously into rats CEA and th PELPK-albumin conjugate were cleared rapidly. The other peptide conjugate and BSA cleared at a much slower rate. Activity of 125I-labeled CEA and PELPK-albumin conjugate per gram of tissue was highest for the liver and spleen. Clearance of 125I-CEA was inhibited by the presence of higher concentrations of the PELPK-albumin conjugate. With isolated rat Kupffer cells, only CEA and the PELPK-albumin conjugate were bound and internalized in vitro and CEA binding was inhibited by higher concentrations of the PELPK-albumin conjugate. Similarly, binding of the PELPK-albumin conjugate was inhibited by the presence of unlabeled CEA. Use of a heterobifunctional cross linking agent demonstrated reaction of the PELPK-albumin with an 80-kDa protein on the Kupffer cell surface by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). This semisynthetic ligand (PELPK-albumin) allows us to examine the function of the 80-kDa receptor without interference due to other properties of CEA including its ability to bind lectins and to cause homotypic aggregation of cells. The consequences of CEA binding to the 80-kDa receptor may have implications in the development of hepatic metastasis from colorectal cancer.

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Internalization and recycling of activated thrombin receptors.

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Shortly after activation by either thrombin or the tethered ligand domain peptide SFLLRN, thrombin receptors undergo homologous desensitization, temporarily losing their ability to respond to both agonists. We have examined the role of receptor internalization and recycling in this process using receptor-directed antibodies as probes. The results show within 1 min c activation > 85% of the approximately 200,000 thrombin receptors on megakaryoblastic human erythroleukemia (HEL) and CHRF-288 cells are sequestered into endosomes via coated pits, after which the majority are transferred to lysosomes. This process does not require proteolysis of the receptor and occurs with sufficient speed to play a major role in the regulatio of thrombin receptor function. Although most of the internalized receptors ar ultimately degraded, approximately 25% return to the cell surface. These recycled receptors are in a state in which they can respond to SFLLRN but not thrombin; nor do they self-activate despite the apparent continued presence of the tethered ligand. In contrast to other G protein-coupled receptors, which are internalized and then recycled in an activatable state, recovery of the thrombin response occurs only after the expression on the cel surface of adequate numbers of newly synthesized receptors.

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